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Misidentification of *Vespula alascensis* as *V. vulgaris* in North America (Hymenoptera: Vespidae; Vespinae)

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ABSTRACT

Morphological and molecular-based comparisons of the North American yellowjackets identified as *Vespula vulgaris* (Linnaeus, 1758) with samples from other parts of the world demonstrate they are not that species. The name *Vespula alascensis* (Packard, 1870) is applicable to the North American species, new status. *Vespa communis* de Saussure, 1857, *non* von Schrank, 1785, is a synonym of *Vespula maculifrons* (du Buysson, 1905), REVISED SYNONYMY.

INTRODUCTION

Vespa vulgaris was described by Linnaeus (1758) from "Europa," and recorded from North America by Kirby (1837). De Saussure (1853–1858) included North America in the distribution of this taxon in his worldwide monograph, but he later (de Saussure, 1857) decided that the North American specimens were a distinct species, describing them as *Vespa communis*. Du Buysson (1905) recognized *V. communis* in his monograph, but also recorded *V. vulgaris* from North America again, and in this he has been followed by subsequent investigators. Since Bequaert's (1932) synopsis of the North American vespine fauna, these have been treated as species of *Vespula*, with *Vespula maculifrons* (du Buysson) used as the name for *Vespa communis*.

Vespula vulgaris was recorded from New Zealand by Thomson (1923), although it apparently did not become established for 60 years (Donovan, 1984). Donovan (1984) noted differences in worker length and nest size between New Zealand specimens and a sample from the western United States, and stated (p. 426) that this disparity "makes it doubtful if the New Zealand population originated in the western United States." One of us (T.R.G.) later compared molecular sequence data for samples of *V. vulgaris* from

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the United Kingdom, United States and New Zealand, and came to the conclusion (Glare, 2004: 2): "The UK and New Zealand wasps were identical, but the '*V. vulgaris*' from the USA was not the same species." This finding led the other of us (J.M.C.) to investigate whether diagnostic differences could be found in the male genitalia. This turned out to be the case. In the present paper we document both the molecular and morphological differences.

MATERIALS AND METHODS

Dissections

Male specimens in the collection of the American Museum of Natural History were dissected, the genital capsule extracted, cleared slightly in warm lactophenol, and examined in glycerin. Some previously dissected males were also available. North American specimens identified as *V. vulgaris* were from Alaska, Alberta, California, New York, Oregon, and "Hudson Bay Territory." Eurasian specimens were from China, the Czech Republic, England, and "Europe," as well as from New Zealand.

DNA Extraction

Extraction of DNA was successfully performed on a number of wasp samples collected and supplied by New Zealand, Australian, U.S. and U.K. collaborators (table 1). Both adults and larvae were extracted using a modification of the method of Henry et al. (1990). Whole or parts of wasp larvae or adults were ground in liquid nitrogen and 1 ml of TENT (10 mM Tris-HCl [pH 7.4], 25 mM EDTA, 10 mM NaCl, and 0.5% Triton X-100) buffer added. After 5 minutes of centrifugation, the pellet was resuspended in 600 µl TEN (10 mM Tris-HCl [pH 7.4], 25 mM EDTA, 10 mM NaCl) buffer, 0.2 mg/ml of proteinase K (Invitrogen Life Technologies), and a final concentration of 1% SDS. Following 4 h incubation at 37°C, 60 µl of 5 M NaCl was added and the DNA twice extracted with an equal volume of phenol-chloroform. The DNA was precipitated with isopropanol, resuspended in 50 µl sterile distilled water, and stored at 4°C or frozen for long-term storage. The optimal concentration of DNA for use in subsequent PCRs was determined empirically.

Polymerase chain reaction amplification

Polymerase chain reaction (PCR) amplifications were performed in 25 µl volumes containing 0.4 µM of each primer (Invitrogen Life Technologies), 2 µl DNA, 0.2 mg/ml BSA (NEB, Biolab), and the following reagents from ABgene, Innovative Sciences: 200 µM dNTPs, 1× ReddyMix^{TM} PCR buffer, 1.5 mM $MgCl_2$, and 0.6 U Thermoprime Plus DNA polymerase. PCR primers which target the mitochondrial region of part of the CO1 gene, using primers mtd6-GGAGGATTTGGAAATTGATTAGTTCC (CI-J-1718 in Simon et al., 1994) and mtd9- CCCGGTAAAATTAAAATATAAACTTC (CI-N-2191 in Simon et al., 1994) were used. PCR products destined for sequencing were amplified in 50 µl volumes containing 0.4 µM of each primer (Invitrogen Life Technologies), 4 µl DNA, 0.2 mg/ml BSA (NEB, Biolab), 200 µM dNTPs (ABgene, Innovative Sciences), 1× buffer containing 1.5 mM $MgCl_2$ (Roche), and 1.75 U Expand High Fidelity PCR system (Roche). Amplifications were carried out in an Eppendorf Mastercycler gradient thermocycler using 30 cycles of 30 sec at 94°C, 45 sec at 54°C, and 1 min at 72°C. Positive (previously successfully amplified DNA) and negative (sterile dH_2O) controls were included in each PCR run.

Sequencing

Expand amplified PCR products were cleaned using a High Pure PCR Product Purification kit (Roche) and sent to the Waikato DNA Sequencing Facility (Hamilton, New Zealand) for sequencing. Genbank numbers for sequences obtained are given in table 1.

Alignment

Sequences were aligned using DNAMAN (Lynnon BioSoft, Quebec, Canada) and ClustalX (Thompson et al., 1997).

Clustering

Ropalidia romandi (Le Guillou) [Polistinae], Genbank accession number AF146677, was used as an outgroup. Symmetric resampling (Goloboff et al., 2003) of the aligned sequenc-

TABLE 1
DNA extractions and Genbank accession numbers

Date received or DNA extracted	Code	Genbank accession no.	Specimen	Locality	Collector
24/03/03	v1	GU207853	*Vespula vulgaris* adult	Pelorus, South Island, NZ	Jo Rees et al., Landcare, New Zealand
24/03/03	v2	GU207855	*Vespula vulgaris* adult	Pelorus, South Island, NZ	
24/03/03	v3	GU207854	*Vespula vulgaris* adult	Pelorus, South Island, NZ	
18/11/98	FQ	GU207851	*V. germanica* (Fabricius) foundress queen	Christchurch, Canterbury, NZ	Nicola Richards, AgResearch, New Zealand
18/12/01	Aw_W1 con	GU207848	*V. germanica* adult	Adelaide?, Australia	Andy Austin, Waite Institute, University of Adelaide
22/07/02	BK2 Aust	GU207850	*V. germanica* adult	Brukunga, Australia	
22/07/02	GRL	GU207852	*V. germanica* larvae	Botanic Gardens, Australia	
08/04/03	Uk vul13	GU207849	Supplied as extracted DNA from *V. vulgaris*	UK	Derek Daly, University of Liverpool
08/04/03	Uk vul16	GU207857			
27/01/03	RC_mac	GU207856	*Vespula maculifrons* adult	Antrim Co., Michigan, USA, Au Sable Institute, Aug.15 2001 AAIB-baited trap	Hal C. Reed, Oral Roberts University
27/01/03	USA mac2	GU207859	*Vespula maculifrons* adult	Tulsa Co., Oklamhoma, USA. Oral Roberts U. campus, Aug.27 2001 AAIB-baited trap	
27/01/03	USA V flav	GU207858	*V. flavopilosa*	Antrim Co., Michigan, USA, Au Sable Institute, Jul.25 2001 AAIB-baited trap.	
27/01/03	USA vul1	GU207860	"*V. vulgaris*"	Antrim Co., Michigan, USA, Au Sable Institute, Jul.25 2001 AAIB-baited trap.	
27/01/03	USA vul2	GU207861	"*V. vulgaris*"	Antrim Co., Michigan, USA, Au Sable Institute, Jul.25 2001 AAIB-baited trap.	

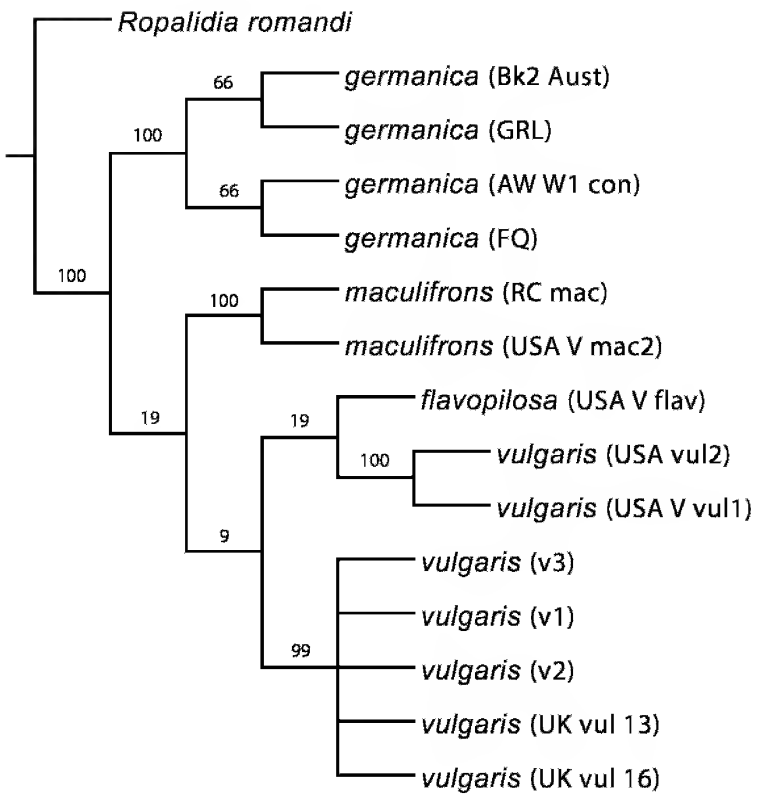


Fig. 1. Symmetric resampling tree generated with TNT, based on approximately 400 bp sequences of the CO1 gene of mitochondrial DNA. Values displayed are frequency differences.

es was carried out with the program TNT (Goloboff et al., 2008), using 10,000 replicates.

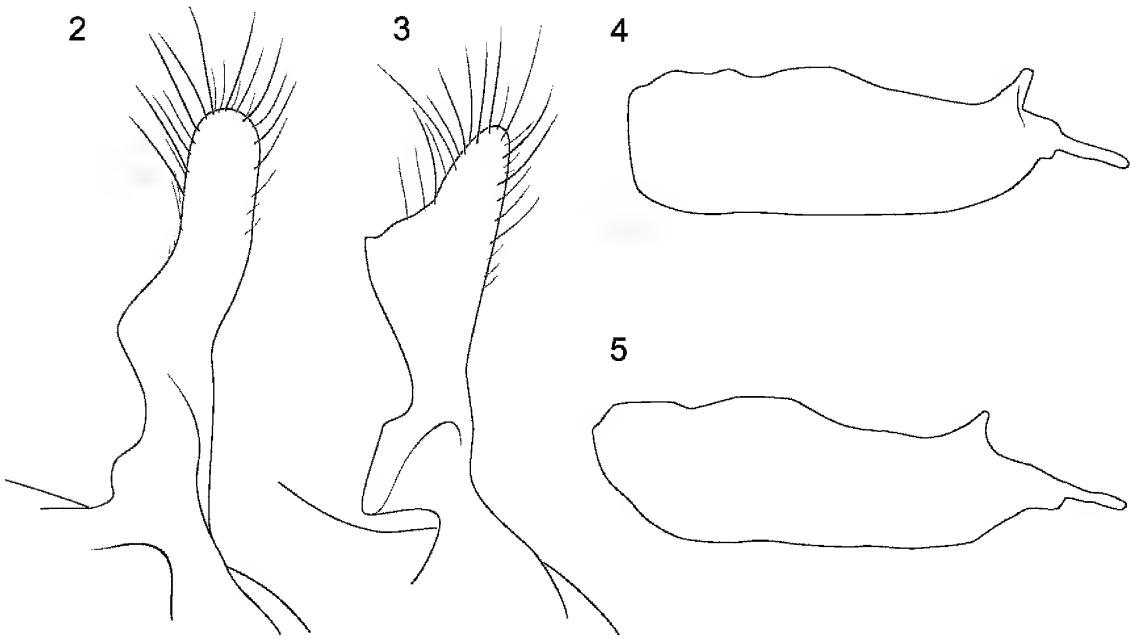
RESULTS

Figure 1 is the tree resulting from symmetric resampling. The North American specimens identified as *V. vulgaris* group with *Vespula flavopilosa* Jacobson rather than with European and New Zealand *V. vulgaris*. North American males are readily distinguished from Eurasian and New Zealand males by characters of the genitalia. The digitus of *V. vulgaris* (fig. 2) is smoothly rounded and symmetrical toward the apex, whereas in the North American form (fig. 3) the digitus is asymmetrical toward the apex. In *V. vulgaris* (Fig. 4) the paramere is somewhat more emarginate beside the dorsal terminal process, so that the process appears slightly longer, than in the North American form (fig. 5; it may be necessary to rotate the paramere through several views to appreciate the difference).

TAXONOMY

The North American species identified as *V. vulgaris* is clearly not that species. The question now is whether there is a name available for the North American species.

Bequaert (1932) treated *Vespa communis* de Saussure, described from "America septentr.," as a synonym of *Vespula vulgaris*, although he had earlier (Bequaert, 1930) treated it as a synonym of *Vespula maculifrons*. He did not state reasons for either synonymy, although presumably the latter followed Rohwer (1926). The synonymy of *communis* with *vulgaris* was followed in the checklist of Vespinae by Carpenter and Kojima (1997). However, J.M.C. has seen type material of *communis* in the Muséum National d'Histoire Naturelle in Paris, and the taxon should be treated as a



Figs. 2–5. Male genitalia. Figs. 2–3, digitus in ventral view: 2, *V. vulgaris*. 3, *V. alascensis*. Figs. 4–5, paramere with dorsal terminal process in dorsal view: 4, *V. vulgaris*. 5, *V. alascensis*.

synonym of *maculifrons*, revised synonymy. In any case, *Vespa communis* de Saussure, 1857, is a junior primary homonym of *Vespa communis* von Schrank, 1785, as was pointed out by Rohwer (1926).

Bequaert (1932) also listed *Vespa alascensis* Packard, 1870, described from "Lower Yukon," as a synonym of *Vespula vulgaris*. Packard (1870) is an obscure publication indeed, because most of the copies of the publication were destroyed in the Great Chicago Fire of 1871. The publication was overlooked until Banks (1920) called attention to a reprint in the Museum of Comparative Zoology. Presumably Bequaert consulted this copy, as the whereabouts of the type of *V. alascensis* are unknown. The description and figure do allow recognition of this taxon, a conclusion also reached by Jacobson et al. (1978). Packard (1870: 27) mentioned eyes reaching to the base of the mandibles, yellow markings, no markings on the scutum, black antennae, an upper and lower yellow spot on the gena the only markings on the orbits, and metasomal apical bands on terga II–IV with "the middle sinus triangular, the outer rounded." Comparing these features with the key in Akre et al. (1981) leads to the couplet for *V. vulgaris*. Other details of the coloration mentioned in the description match *V. vulgaris* as well, viz. "the indentation in the eyes yellow" [evidently referring to the clypeus], "with a central broad black band, dilated below into a rounded, larger termination," "Mandibles yellow, cutting edge and teeth black" and mesonotum "with the usual oblique line in front of the wings, and the triangular spot beneath; two yellow spots on the scutellum, and two lunate yellow spots behind." The illustrations of the variation in *V. vulgaris* in Jacobson et al. (1978) and Eck (1999) show all these details. According to Eck (1999) North American specimens as a rule are darker than Eurasian *V. vulgaris*, and in particular never have yellow markings on the propodeum, and none are mentioned in the description of *alascensis*. However, this feature may have little diagnostic value. Eck (1999) illustrated Palearctic *vulgaris* lacking such spots, and Miller (1961: 10) mentioned propodeal spots present in a small fraction of colony populations (<5%), although Jacobson

et al. (1978) and Eck (1999) observed only black propodea in North American *vulgaris*.

The only point in Packard's description that is difficult to evaluate is the characterization of the clypeus as "deeply cleft," however the figure shows nothing unusual. The description compared *alascensis* to the new species *Vespa tripunctata*, now considered a synonym of *Vespula austriaca* (Panzer). The description of *tripunctata* mentioned "Clypeus with two prominent sharp black teeth on the front edge" (Packard, 1870: 26), and sharply produced apical angles of the clypeus are indeed characteristic of vespine social parasites, such as *V. austriaca*. Packard's statement about the clypeus of *alascensis* may have meant to contrast it with that of *tripunctata*, in lacking sharp apical angles.

The identification of *alascensis* as North American *V. vulgaris* is therefore accepted here, and that name is available for the distinct species: *Vespula alascensis* (Packard), NEW STATUS.

DISCUSSION

Five Holarctic species were recognized in the checklist of Vespinae by Carpenter and Kojima (1997); now there are four. In each case, names are available for both the palearctic and nearctic forms, as these were described as separate species. These are *Vespula rufa* (Linnaeus) with *V. intermedia* (du Buysson) its Nearctic synonym, *V. austriaca* with *V. infernalis* (de Saussure) in the Nearctic, *Dolichovespula norwegica* (Fabricius) with *D. albida* (Sladen) in the Nearctic, and *D. adulterina* (du Buysson) with *D. arctica* (Rohwer) in the Nearctic. In view of our present findings it would seem that reinvestigation of the status of all these forms is warranted.

Another Holarctic species was recognized by Matsuura and Yamane (1990) and Pekkarinen (1995), who treated the nearctic *Dolichovespula norvegicoides* (Sladen) and the palearctic *D. pacfica* (Birula) as subspecies of *D. norvegicoides*. In this case, a diagnostic difference between *D. norvegicoides* and *D. pacifica*, the elevation between gena and clypeus, was adduced by Archer (1989) (and confirmed by Pekkarinen, 1995). At present only the characters of the male genitalia reliably diagnose *Vespula vulgaris* and *V. alascensis*. Females of *V. alascensis* are generally said to be darker than *V. vulgaris* (Eck, 1999), but there is overlap. Even the propodeal spots, often present in *V. vulgaris* and absent in *V. alascensis*, can be absent in *V. vulgaris* (see figures in Eck, 1999). But the matter should be further investigated: the character differentiating *D. norvegicoides* and *D. pacifica* is a subtle one, and a similar feature may yet be found to diagnose *V. vulgaris* and *V. alascensis*.

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